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Platelet Count, Platelet Function, Coagulation Activity and Fibrinolysis in the Acute Phase of Inflammatory Bowel Disease

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Summary: Twenty two patients with exacerbation of inflammatory bowel disease (19 with *Crohn*'s disease, 3 with ulcerative colitis) and thrombocytosis were tested for possible activation of the coagulation and platelet system.

Fifteen patients had abnormal platelet function i.e. unphysiologically high sensitivity in vitro towards ADP 2 µmol/l aggregation induction. In 81.8% of the patients we found enhanced fibrinogen concentrations. In 22.7% of the patients thrombin-antithrombin III values exceeded the upper limit of the reference range, and in 68.2% of the patients the D-Dimer concentration exceeded the upper reference limit as a result of reactive fibrinolysis. The altered platelet count and function, and the increased levels of fibrinogen and thrombin-antithrombin III with reactive fibrinolysis activation indicate the presence of prethrombotic factors in patients with exacerbation of inflammatory bowel disease. The presence of enhanced fibrinolysis in these patients might have consequences for the therapeutic treatment.

Introduction

As we know from the literature (1–3) thromboembolism is an accepted complication of inflammatory bowel disease. In the acute phase, F VIII, F II, fibrinogen, plasminogen, prekallikrein and high molecular weight kallikrein are enhanced, which could account for thromboembolic complications (4–7). No evidence has been reported, however, for a prethrombotic state in the stable phase of chronic inflammatory bowel disease (8). Recent research on the changes in parameters of the fibrinolysis system in the peripheral blood of patients with inflammatory bowel disease has shed some light on the nature and magnitude of the disturbances of fibrinolysis and their relationship (9) to the development of deep venous thrombosis. There is evidence for a decrease in tissue plasminogen activator as well as an increase in plasminogen activator inhibitor, both effects being possible explanations for the known risk of thromboembolic complications in these patients (9, 10). Moreover, there are also reports on the occurrence of thrombocytosis in the exacerbation phase of inflammatory bowel disease. Until the present work, however, no data were available on the functional state of platelets in the acute phase of inflammatory bowel disease. We therefore investigated the in vitro aggregation activity of the thrombocytes. For the investigation of the state of the coagulation system we determined fibrinogen, the fibrin monomer concentration and the thrombin-antithrombin III complex. For the investigation of fibrinolysis we chose the recently developed ELISA-assay for the D-Dimer.

Materials and Methods

Patients

Plasma samples were collected from 22 patients (16 females, 6 males, tab. 1) with histologically proven intestinal bowel disease, who visited the outpatient clinic for gastroenterology and presented themselves for acute exacerbation of the disease. The patient group consisted of 19 patients with *Crohn*'s disease and

Tab. 1. Basic characteristics of the patients

	n	Age (a) $\bar{x}$	± s
Overall Age	22	36.5	15.9
Age males	6	37.2	15.8
Age females	16	36.3	15.9

3 patients with ulcerative colitis. The activity index of *van Hees* (11) ranged from 210–313. The use of medication was excluded. All the female patients were using oral contraceptive drugs. The main inclusion criterium from the laboratory point of view was the presence of a thrombocytosis. A reference group consisted of subjectively healthy individuals from the hospital and laboratory staff.

Samples

For the platelet count, venous blood was collected in plastic vials containing 1.6 g/l EDTA-K_2 (Sarstedt, Nümbrecht, FRG). Samples were kept between 30 min and 4 hours at room temperature until analysis.

Coagulation parameters were determined in citrated plasma, prepared by centrifugation of nine volumes freshly drawn blood with one volume trisodium citrate (0.11 mol/l) for 10 min (1600 *g*) at 25 °C. The plasma was stored at −70 °C in plastic tubes and thawed with tap water for 5 min before serial analysis.

The platelet aggregation test was performed on platelet rich plasma, which was prepared by immediate centrifugation of citrated blood (prepared as described above) at 200 *g* for 10 minutes at room temperature. After gentle aspiration of the platelet-rich plasma, using a plastic pipette, the remaining blood was centrifuged at 2000 *g* for 10 min at room temperature; the resulting platelet-poor plasma was aspirated and subsequently centrifuged at 10000 *g* at 4 °C for 10 min to obtain platelet-free plasma. Platelet-rich plasma and platelet-free plasma were used for standardization of the end concentration of the platelet count in the test. The collagen-induced aggregation was performed at a thrombocyte concentration of 400 × 10^9/l, whereas the other induced aggregations were performed at a platelet concentration of 200 × 10^9/l. The spontaneous (i.e. induced by stirring alone) aggregation was carried out in platelet-rich plasma.

Methods

Platelets were counted with a Sysmex E-4000 (Kobe, Japan) cell counter. Platelet aggregation was measured by a turbidimetric method using a Daiichi dual channel aggregation device. The test concentrations of the different inducing agents are quoted in the results. Fibrinogen was determined by the clotting assay of *Clauss*. The FM test is a semi-quantitative test from the Boehringer Mannheim Corp (FRG), using fibrinogen-coated erythrocytes to detect circulating fibrin monomers in plasma (detection level 10 mg/l). Thrombin-antithrombin III was determined with an ELISA test kit (Behring Corporation, Marburg, FRG). D-Dimer was assayed in plasma with an ELISA method (Boehringer Mannheim, FRG).

Results

Values for the coagulation and fibrinolysis parameters are presented in table 2. Close agreement between the mean and median value was observed only in the case of fibrinogen (5.6 and 5.8 g/l respectively). For the platelets, thrombin-antithrombin III and D-Dimer the mean values are higher than the median values (platelets 575/548 × 10^9/l, thrombin-antithrombin III 3.4/2.0 µg/l, D-Dimer 1101/630 µg/l). The range of the values is relatively large for all parameters; in particular, the maximal D-Dimer value is 70 times higher than the minimal value. The range for the platelets was 400–997 × 10^9/l, for fibrinogen 3.0–9.0 g/l, for thrombin-antithrombin III 1.0–10.8 µg/l, and for D-Dimer 100–7000 µg/l.

Table 3 shows the percentages of values exceeding the upper limits of the respective reference ranges, i.e. fibrinogen 81.2%, the FM test 4.5%, thrombin-antithrombin III concentration 22.7% and D-Dimer concentration 68.2%.

Table 4 compares the mean values of parameters for the patients and the reference group. On the 95% level the patient and reference values for fibrinogen

Tab. 2. Basic characteristics of the parameters investigated

Parameter	Mean	Minimum	Median	Maximum
Platelets (10^9/l)	575	400	548	997
Fibrinogen (g/l)	5.6	3.0	5.8	9.0
Thrombin-antithrombin III (µg/l)	3.4	1.0	2.0	10.8
D-Dimer (µg/l)	1101	100	630	7000

Tab. 3. Comparison of parameter values for patients with the reference ranges

Parameter	Reference range	Mean	SD	% of reference range
Platelets	130 –400 × 10^9/l	575	158	100
Fibrinogen	1.7– 4.0 g/l	5.6	1.5	81.8
Fibrin monomers	⩽ 10 nmol/l	–	–	4.5
Thrombin-antithrombin III	1.0– 4.1 µg/l	3.4	2.9	22.7
D-Dimer	0 –450 µg/l	1101	1528	68.2

Tab. 4. Comparison of the differences of the means with the *Student* t-test

Parameter	Reference group		Patient group		Significance p*
	Mean	SD	Mean	SD	
Platelets (10^9/l)	265	67.5	575	158	<0.0001
Fibrinogen (g/l)	2.85	0.58	5.8	1.5	<0.0001
Thrombin-antithrombin III (µg/l)	2.55	0.78	3.4	2.9	>0.05 <0.10
D-Dimer (µg/l)	238	100	1101	77.8	<0.0001

* *Student* t-test

and D-Dimer were significantly different, whereas the thrombin-antithrombin III concentrations were not.

In table 5 the results are classified according to sex. The platelet counts were lower in males than in females (531/592 × 10^9/l), as were the fibrinogen concentrations (4.3/6.1 g/l); for the other parameters the relationship between males and females was reversed: thrombin-antithrombin III 5.7 vs. 2.6 µg/l, D-Dimer 2103 vs. 699 µg/l, and ADP 2 µmol/l aggregation 81.7 vs. 76.8%.

In the case of the platelets, fibrinogen, thrombin-antithrombin III and D-Dimers, the sex differences were statistically significant. Table 6 shows the comparison of values for male patients and for female patients with those for the reference group. Highly significant differences were found between the mean values of parameters from male or female patients and those of the reference group, with the exception of the thrombin-antithrombin III levels in female patients.

Tab. 5. Comparison of the parameters values for males and females

Parameter	Males		Females		Significance* P-values
	Mean	SD	Mean	SD	
Platelets (10^9/l)	531	107	592	173	<0.05
Fibrinogen (g/l)	4.3	1.0	6.1	1.4	<0.05
Thrombin-antithrombin III (µg/l)	5.7	4.2	2.6	1.9	<0.05
D-dimer (µg/l)	2103	2680	699	411	<0.05
ADP 2 µmol/l aggregation (A_{max} in %)	81.7	4.7	76.8	14.4	>0.05

* *Student* t-test

Tab. 6. Comparison of the parameters values for males and females with those of the reference group

Parameter	Reference group G1		Male patients G2		Female patients G3		Significances* P-values	
	Mean	SD	Mean	SD	Mean	SD	G2/G1	G3/G1
Platelets (10^9/l)	265	67.5	531	107	592	173	<0.0001	<0.0001
Fibrinogen (g/l)	2.85	0.58	4.3	1.0	6.1	1.4	<0.0001	<0.0001
Thrombin-antithrombin III (µg/l)	2.6	0.8	5.7	4.2	2.6	1.9	<0.0001	>0.05
D-dimer (µg/l)	238	100	2103	2680	699	411	<0.0001	<0.0001
ADP 2 µmol/l) aggregation (A_{max} in %)	30	11.0	81.7	4.7	76.8	14.4	<0.0001	<0.0001

* *Student* t-test

Tab. 7. The criteria for normality and the results of the different types of aggregation tests

Aggregation inducer	Criteria for normality		Normal	Abnormal	Total
	Aggregation pattern	Aggregation maximum (Change in light transmission)	Number (n)	Number (n)	(n)
ADP (µmol/l)					
0.2	AA	0	19	3	22
2	R	⩽50	7	15	22
10	NR	>50	22	0	22
Stirring (spontaneous)	AA	0	21	1	22
Collagen	NR	>50	22	0	22
Ristocetin	NR	>50	22	0	22
Epinephrine	NR	>50	22	0	22

AA = aggregation absent NR = non reversible R = reversible

Tab. 8. Comparison of the ADP 2 µmol/l aggregation of the control and the patient group

	ADP aggregation (2 µmol/l)						Significance level*
	Reversible (R)			Non reversible (NR)			
	A_{max} ($\bar{x}$ in %)	SD (%)	n	A_{max} ($\bar{x}$ in %)	SD (%)	n	P
Control group of healthy individuals	30	11	49	–	–	–	–
Patient group without treatment in phase of exacerbation	35	7	7	77.5	13.9	15	<0.0001

* *Student* t-test

Table 7 summarizes the results and the criteria for normality of the different aggregation tests carried out in this study. We found no abnormality for the collagen, ristocetin, epinephrine or ADP 10 µmol/l platelet aggregation (0/22). One patient however presented with spontaneous aggregation, three out of fifteen with 0.2 µmol/l ADP aggregation and fifteen of the twenty two patients with increased 2 µmol/l ADP aggregation. In table 8 we can see that the maximal amplitude (A_{max}) of the 2 µmol/l ADP aggregation of the normal patient group with an A_{max} of 35% hardly differs from the A_{max} of the control group of healthy individuals (A_{max} = 30%). The difference however between the abnormal patient group (A_{max} = 77.5%) and the normal patient group was significant ($p < 0.0001$).

Discussion

This study was designed to investigate the basis of the thrombocytosis in patients with *Crohn*'s disease and colitis ulcerosa, which is known to occur in patients with exacerbation of the disease (1, 12, 13).

From the results of this study it seems clear that the degree of thrombocytosis is greater for females than for males, a fact that is consistent with earlier reports (14) of higher platelet counts for females in a reference population. The question arises as to the degree of thrombocytosis that can be considered as a prethrombotic factor. For this purpose we investigated platelet aggregation after induction with different stimuli. One patient showed spontaneous aggregation, three patients showed aggregation with 0.2 µmol/l ADP, and fifteen patients showed enhanced aggregation with 2 µmol/l ADP. Taking account of the need for care in the handling and interpretation of aggregation tests, we can conclude that the platelets of 15/22 patients showed an unphysiologically strong in vitro reaction in the ADP 2 µmol/l aggregation induction test, which can be considered as representing a prethrombotic state. This is in agreement with the observations of *Lake* et al. (4), who reported increased platelet counts associated with abnormal platelet function in patients with bowel disease.

The platelet aggregation effect was of the same magnitude in males and females. This was not the case for fibrinogen, which showed a significant difference between males and females, and a significant increase in comparison with the reference group. *Lee* et al. (7) reported similar changes in fibrinogen in chronic ulcerative colitis. It is conceivable that high levels of fibrinogen may increase the risk of thrombus formation. Moreover, we know that elevation of fibrinogen increases blood viscosity, which may in turn further enhance the risk of thrombus formation.

In summary, it seems that, in our patient group, fibrinogen can be considered as a risk factor for the development of thrombosis, and to a greater degree in women than in men. In 22.7% of the patients, the increased thrombin-antithrombin III concentrations suggest the possibility of clotting activation, although the difference between the mean of the reference and the patient group is not significant on the 95% level. When the thrombin-antithrombin III results were classified according to sex, the mean for males, but not that for females, showed a significant difference. *Ghosh* et al. (15) described patients with inflammatory bowel disease, decreased antithrombin III and a history of thrombosis, and speculated that their results were due to an increased consumption of antithrombin III. It can be concluded that coagulation activation occurs, at least in the male patients. This was clearly established by the results of the D-Dimer analysis. Both the fact that 68.2% of the overall results exceed the upper reference limit and the fact that the overall mean of the D-Dimer determinations was significantly enhanced in the patient group in relation to the reference group can be taken as evidence for the presence of a reactive fibrinolysis after preceeding coagulation in these patients. This is also suggested by the recent findings of *Wakefield* et al., who reported that *Crohn*'s disease is mediated by multifocal gastrointestinal infarction (19). This is important for understanding the pathogenesis of *Crohn*'s disease and its common clinical features. The strongest fibrinolysis effect was again found in the male patients, which is in full agreement with the previously mentioned

thrombin-antithrombin III results for males and females. For the rest, it seems inconceivable at first sight, that the degree of fibrinolysis would be somewhat higher than expected from the thrombin-antithrombin III results. An acceptable explanation, however, could be that all the reactions caused by thrombin generation will ultimately result in the production of D-Dimers.

Increased fibrinolytic activity was first described by *Kwaan* et al. (16, 17) in patients with active colitis, and *Kondo* et al. (18) later reported the same results. These investigators suggested some connection between hyperfibrinolysis and anal blood loss, and reported clinical improvement and reduction of blood loss after antifibrinolytic therapy.

In summary, our study suggests the presence of prethrombotic factors in the acute phase of inflammatory bowel disease, which might contribute to an increased risk of thrombosis, as also confirmed by de *Jong* et al. (9). The activation of the fibrinolysis system in the acute phase of inflammatory bowel disease, as shown indirectly in this study by measurement of the D-Dimers, must be the result of increased plaminogen transformation. Plasmin is known to have – in addition to its main fibrinolytic function – the ability to activate collagenases, which in excess could adversely affect wound healing, a process undoubtedly needed in these patients. Thus, it may be worth reinvestigating and reconsidering the treatment of these patients with antifibrinolytic agents.

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